

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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To:

GREAVES, Carol, Pauline
Greaves Brewster
Indigo House, Cheddar Business Park
Wedmore Road, Cheddar
Somerset, BS27 3EB
GRANDE BRETAGNE

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Entered

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Date of mailing
(day/month/year)

08.12.2004

Applicant's or agent's file reference
CPGP/205/WOD

IMPORTANT NOTIFICATION

International application No.
PCT/GB 03/04412

International filing date (day/month/year)
10.10.2003

Priority date (day/month/year)
10.10.2002

Applicant

THE SECRETARY OF STATE FOR DEFENCE et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465


Authorized Officer

Digiusto, M

Tel. +49 89 2399-8162



PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference CPG/P/205/WOD	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB 03/04412	International filing date (<i>day/month/year</i>) 10.10.2003	Priority date (<i>day/month/year</i>) 10.10.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant THE SECRETARY OF STATE FOR DEFENCE et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 8 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the opinion</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input checked="" type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the international application</p>		
Date of submission of the demand 29.04.2004	Date of completion of this report 08.12.2004	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hennard, C Telephone No. +49 89 2399-7355	



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB 03/04412

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-26 as originally filed

Claims, Numbers

1-41 received on 22.11.2004 with letter of 22.11.2004

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

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5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	4-5, 13-16, 23, 27, 29, 33, 40-41
	No: Claims	1-3, 6-12, 17-22, 24-26, 28, 30-32, 34-39
Inventive step (IS)	Yes: Claims	None
	No: Claims	1-41
Industrial applicability (IA)	Yes: Claims	1-41
	No: Claims	None

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and /or

2. Non-written disclosures (Rule 70.9)

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/04412

Re Item I

Basis of the report

The amendment concerned concerns claim 1 in it's entirety for the following reasons:

Claim 1 as filed concerned a method for detecting the presence of a target nucleic acid sequence in a sample characterised by the use of a fluorescently labelled probe and a DNA duplex binding agent which can absorb fluorescent energy but which does not emit visible light. This feature is also disclosed in the description on page 4, lines 3-9 where it is stipulated that the DNA duplex binding agent should not emit light in the visible range (390nm to 750nm) in order to avoid signal overlap with the signal of the probe.

In the newly filed **claim 1** the feature relating to the DNA duplex binding agent has been amended as follows: "said DNA duplex binding agent being one which does not emit visible light during this method". The new formulation of the claim allows the use of DNA duplex binding agents which emit light in the visible range under certain circumstances as long as they do not emit visible light under the conditions of the method.

Page 7, lines 23-33 has been cited as a basis for the modification, but this passage only refers to the fact that the DNA duplex binding agent must "not impede the progress of the amplification reaction". Further, page 4, line 36 - page 5, line 2 was cited as basis for the modification, but the sentence before that passage clearly states that the agent in question can emit radiation at wavelengths outside the visible range, e.g. infrared range. These passages can not be seen as basis for the modification of the claim.

Therefore, in the absence of a basis for this modification, and since it deviates from the general teaching of the application as filed, this amendment is considered as an extension of the scope of the application as filed which is contrary to the requirements of **article 34(2)(b) PCT**.

Therefore, this IPER is based on claim 1 as filed with letter of 08.09.2004 and claims 2-41 as filed with letter of 22.11.2004.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: EP-A-0 699 768

D2: EP-A-0 872 562

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB 03/04412

D3: WO-A-02 097132
D4: US-A-5, 208, 323
D5: US2002/01066882 A
D6: US-A-5, 858, 397

2. Novelty (Article 33(2) PCT):

D1 (claims 5-6, page 3, line 55 - page 4, line 8; page 6, lines 34, 41-42 and 47-52) discloses a method for detecting a target nucleic acid in a sample using a DNA binding compound which can be daunomycin, and a labelled oligonucleotide probe. The label is fluorescent and is quenched by the DNA binding agent. The method of **D1** further involves PCR amplification and fluorescent measurements of the sample. **D1** discloses therefore the subject-matter of the independent **claims 1 and 24** of the present application.

The use of the DNA binding agent (independent **claim 35**) in detecting a target nucleic acid are also considered disclosed in **D1**.

In the light of **D1**, **claims 1-3, 6-12, 17-22, 24-26, 28, 35-39** are not new.

D4 (example 1) discloses a mixture containing daunorubicin (also known as daunomycin) in a phosphate buffer. This disclosure affects the novelty of **claims 30-32 and 34** of the present application.

D5 (claim 5; figures 6) disclose mixtures of DNA with nogalamycin. This document further discloses the use of such intercalating agents to detect the presence of a target DNA in a sample (claim 1). Therefore **D5** is affecting the novelty of **claims 30-32 and 34-39** of the present application.

D6 (column 9, lines 46-50) discloses mitoxantrone in a buffer and is affecting the novelty of **claims 30-32 and 34**.

In order to summarise the above objections, **claims 1-3, 6-12, 17-22, 24-26, 28, 30-32, 34-39** of the present application are not new and do not fulfil the requirements of **article 33(2) PCT**.

3. Inventive merit (Article 33(3) PCT):

D1 (see the above mentioned passages), which is considered to be the closest prior art, discloses a method for detecting the presence of a target nucleic acid in a sample involving a fluorescent probe capable to hybridise to the target and a duplex binding agent capable of quenching the fluorescence of the probe.

The method of the present **claim 4** of the application distinguishes itself from **D1** by the use of a specific DNA binding agent, namely mitoxantrone.

The problem to be solved by the present application is to provide a new method for detecting a target nucleic acid in a sample.

The solution suggested by the present application is considered not to involve an inventive merit since it is expected from the skilled person to replace a DNA binding agent (daunomycin) by an other agent known in the art to intercalate in duplex DNA and having a close structure to the compound known from **D1** to solve the problem. In the absence of any surprising effect obtained by the use of the specific compound, no inventive merit can be found in this feature.

The other features disclosed in the claims not affected by the novelty objection are also not considered to involve an inventive merit since they consist in well known adaptations of the method in the art of detecting nucleic acid and do not demonstrate any unexpected effect.

It is concluded that **claims 4-5, 13-16, 27, 29, 33 and 40-41** do not fulfil the requirements of **article 33(3) PCT**.

4. Industrial applicability (Article 33(4) PCT):

Due to the nature of the claims, an industrial applicability of the invention is obvious and **claims 1-41** of the present application are considered to fulfil the requirements of **Article 33(4) PCT**.

5. The following items require the applicant's attention:

5.1 The feature "which does not emit visible light" found in **claim 1** to characterise the DNA duplex binding agent is unclear since according to preferred embodiments of the claims, daunomycin possesses this feature (in **claim 3** of the present application, daunomycin is claimed as a preferred DNA duplex binding agent which does not emit visible light). Daunomycin is also described in the prior art **D2** (page 8, lines 29-33) as an alternative to the intercalating fluorescent dye ethyidium bromide which is known to emit visible light. Therefore, this feature is considered to render the scope of protection of the claims unclear (**Article 6 PCT**).

5.2 **Claim 38** is unclear due to the feature "functional group" which in the present context has no clear technical meaning. This claim does not fulfil the requirements of **Article 6 PCT**.

5.3 The feature (iii) of **claim 30** is unclear since it can encompass any kind of reagent. In order for this claim to satisfy to the requirements of **Article 6 PCT**, the definition of the reagent must be introduced in the claim.

5.4 Contrary to the requirements of **Rule 5.1(a)(ii) PCT**, the relevant background art disclosed in **D1 and D2** is not mentioned in the description, nor are these documents identified therein.

5.5 **D3** is an intermediate document published on 05.12.2002, between the priority

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. . PCT/GB 03/04412

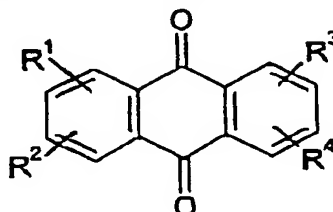
and filing date of the present application (**Rule 70.10 PCT**). Therefore **D3** is not considered to constitute prior art for the present application during the international phase. **D3**, having a filing date of 24.05.2002, would become relevant with respect to novelty and inventive step during the national/regional phase to come if the priority of the present application is not valid. Moreover, **D3** will constitute prior art under the terms of **Article 54(3) EPC** for all the subject-matter of the application when entering the national/regional phase before the EPO.

- 5.6 Dependency of claim 34 is incorrect, it should be dependent from claim 30 to 33 (**Article 6 PCT**).

Claims

1. A method for detecting the presence of a target nucleic acid sequence in a sample, said method comprising:
- 5 (a) adding to a sample suspected of containing said target nucleic acid sequence, a fluorescently labelled probe specific for said target sequence, and a DNA duplex binding agent which can absorb fluorescent energy from the fluorescent label on the probe but which does not emit visible light,
- 10 (b) subjecting the thus formed mixture to an amplification reaction in which target nucleic acid is amplified,
- (c) subjecting said sample to conditions under which the said probe hybridises to the target sequence, and
- (d) monitoring fluorescence from said sample.
- 15
2. A method according to claim 1 wherein the DNA duplex binding agents has a fused conjugated ring system.
3. A method according to claim 1 or claim 2 wherein the DNA
- 20 duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or its salt such as the hydrochloride or dihydrochloride salt, or nogalamycin (2R-(2 α ,3 β ,4 α ,5 β ,6 α ,11 β ,13 α ,14 α)]-11-[6-deoxy-3-C-mehtyl-2,3,4-tri-O-methyl- α -L-mannopyranosyl)oxy]-4-
- 25 (dimethylamino)-3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-14-carboxylic acid methyl ester).
4. A method according to claim 3 wherein the DNA binding
- 30 agent is mitoxantrone.

5. A method according to claim 1 or claim 2 wherein the DNA binding agent is a compound of formula (I)



(IA)

5 wherein R¹, R², R³ and R⁴ are independently selected from hydrogen, X, NH-ANHR and NH-A-N(O)R'R'' where X is hydroxy, halo, amino, C₁₋₄alkoxy or C₂₋₈alkanoyloxy, A is a C₂₋₄alkylene group with a chain length between NH and NHR or N(O)R'R'' of at least 2 carbon atoms and R, R' and R'' are each independently
10 selected from C₁₋₄alkyl and C₂₋₄hydroxyalkyl and C₂₋₄dihydroxyalkyl, provided that a carbon atom attached to a nitrogen atom does not carry a hydroxy group and that no carbon atom is substituted by two hydroxy groups; or R' and R''
15 together are a C₂₋₆alkylene group which, with the nitrogen atom to which R' and R'' are attached for a heterocyclic ring having 3 to 7 atoms, with the proviso that at least one of R¹, R², R³ and R⁴ is a group NH-A-N(O)R'R''.

20 6. A method according to any one of the preceding claims wherein the target nucleic acid is rendered single stranded prior to hybridisation to the probe in step (c).

25 7. A method according to any one of the preceding claims wherein the amplification reaction is the polymerase chain reaction (PCR).

8. A method according to any one of the preceding claims wherein the probe hybridises with the target nucleic acid
30 during every cycle of the amplification reaction.

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9. A method according to claim 8 wherein the fluorescence from the sample is monitored throughout the amplification reaction.
- 5 10. A method according to claim 9 wherein fluorescence data generated is used to determine the rates of probe hybridisation.
- 10 11. A method according to any one of claims 8 to 10 wherein the fluorescence data is used to quantitate the amount of target nucleic acid present in the sample.
- 15 12. A method according to any one of the preceding claims wherein the fluorescent label is a rhodamine dye, Cy5, fluorescein or a fluorescein derivative.
- 20 13. A method according to any one of the preceding claims wherein the fluorescent label is attached at an end region of the probe.
- 25 14. A method according to claim 13 wherein the fluorescent label is attached at the 3' end of the probe and prevents extension thereof by a polymerase.
- 30 15. A method according to anyone of the preceding claims wherein the probe is designed such that it is released intact from the target sequence during a phase of the amplification process other than the extension phase.
- 35 16. A method according to any one of claims 1 to 14 wherein the probe is released intact from the target sequence during the extension phase of the amplification process by the action of the polymerase, and the amplification reaction is effected using a polymerase which lacks 5'-3' exonuclease activity.

30

17. A method according to claim 1 which comprises performing nucleic acid amplification on a target polynucleotide in the presence of (a) a nucleic acid polymerase (b) at least one primer capable of hybridising to said target polynucleotide,
5 (c) an oligonucleotide probe which is capable of binding to said target polynucleotide sequence and which contains a fluorescent label and (d) a DNA duplex binding agent which is capable of absorbing fluorescent energy from the said fluorescent label, and which does not emit light in the visible
10 range of the spectrum; and monitoring changes in fluorescence during the amplification reaction.
18. A method according to claim 17 wherein the amplification is suitably carried out using a pair of amplification primers.
15
19. A method according to claim 17 or claim 18 wherein the nucleic acid polymerase is a thermostable polymerase.
20. A method according to any one of the preceding claims wherein in a further step, a hybridisation assay is carried out and a hybridisation condition which is characteristic of the sequence is measured.
21. A method according to claim 20 wherein the condition is
25 temperature, electrochemical potential, or reaction with an enzyme or chemical.
22. A method according to claim 21 wherein the condition is temperature.
30
23. A method according to claim 22 which is used to detect allelic variation or a polymorphism in a target sequence.
24. A method for determining a characteristic of a sequence,
35 said method comprising;

a) adding to a sample suspected of containing said sequence, a fluorescently labelled probe specific for said target sequence and a DNA duplex binding agent able to absorb fluorescence from a fluorescent label on the probe but which does not emit radiation in the visible range of the spectrum,

5 (b) subjecting said sample to conditions under which the said probe hybridises to the target sequence,

(c) monitoring fluorescence from said sample and determining a particular reaction condition, characteristic of said sequence, at which fluorescence changes as a result of the hybridisation of the probe to the sample or destabilisation of the duplex formed between the probe and the target nucleic acid sequence.

25. A method according to claim 24 wherein the reaction condition characteristic of said sequence is temperature, electrochemical potential, or reaction with an enzyme or chemical.

26. A method according to claim 25 wherein the condition is temperature.

27. A method according to any one of claims 24 to 26 wherein the results obtained from two sequences are compared in order to determine the presence of polymorphisms or variations therebetween.

28. A method according to any one of claims 24 to 27 wherein the DNA duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or its salt such as the hydrochloride or dihydrochloride salt or nogalamycin (2R-(2 α ,3 β ,4 α ,5 β ,6 α ,11 β ,13 α ,14 α)]-11-[6-deoxy-3-C-methyl-2,3,4-tri-O-methyl- α -L-mannopyranosyl]oxy]-4-(dimethylamino)-3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-

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6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-14-carboxylic acid methyl ester).

29. A method according to any one of claim 24 to 27, wherein
5 the DNA duplex binding agent is a compound of formula (IA) as defined in claim 5.

30. A kit for use in the method according to any one of the
preceding claims, which kit comprises (i) a DNA duplex binding
10 agent which is able to absorb fluorescent energy but which does not emit radiation in the visible range of the spectrum, and either (ii) a fluorescently labelled probe specific for a target nucleotide sequence, or (iii) one or more reagents necessary for conducting an amplification reaction.

15 31. A kit according to claim 30 which contains (iii) and wherein the reagents are selected from primers, DNA polymerase, buffers, or adjuncts known to improve PCR.

20 32. A kit according to claim 30 or claim 31 wherein the DNA duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or its salt such as the hydrochloride or dihydrochloride salt or nogalamycin (2R-(2 α ,3 β ,4 α ,5 β ,6 α ,11 β ,13 α ,14 α)]-11-[6-deoxy-3-C-
25 methyl-2,3,4-tri-O-methyl- α -L-mannopyranosyl)oxy]-4-(dimethylamino)-3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-14-carboxylic acid methyl ester).

30 33. A kit according to claim 30 or claim 31 wherein the DNA duplex binding agent is a compound of formula (IA) as defined in claim 5.

35 34. A kit according to any one of claims 30 to 33 which comprises both (i) and (ii).

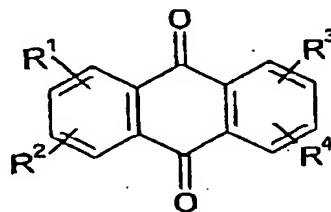
33

35. The use of a DNA duplex binding agent which can absorb fluorescent energy but which does not emit visible light in a method for detecting the presence of a target nucleic acid sequence in a sample by the amplification of said target nucleic acid.

36. The use according to claim 35 wherein the DNA duplex binding agent comprises a conjugated aromatic ring system.

37. The use according to claim 36 wherein the DNA duplex binding agent comprises an anthracyclin or anthraquinone.

38. The use according to any one or claims 35 to 37 wherein the DNA duplex binding agent is an optionally substituted anthraquinone of structure (I)



(I)

where R^1 , R^2 , R^3 and R^4 are independently selected from hydrogen, a functional group, or a hydrocarbyl group optionally substituted by for example functional groups, or R^1 and R^2 or R^3 and R^4 are optionally joined together to form a ring which optionally contains heteroatoms, and/or is optionally substituted by a functional group or a hydrocarbyl group.

39. The use according to any one of claims 35 to 38 wherein the DNA duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or its salt such as the hydrochloride or dihydrochloride salt or nogalamycin (2R-(2 α ,3 β ,4 α ,5 β ,6 α ,11 β ,13 α ,14 α))-11-[6-deoxy-3-C-methyl-2,3,4-tri-

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O-methyl- α -L-mannopyranosyl)oxy]-4-(dimethylamino)-
3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-
6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-
14-carboxylic acid methyl ester).

5

40. The use according to any one of claims 35 to 38 wherein
the DNA duplex binding agent is a compound of formula (IA) as
defined in claim 5.

10 41. The use according to claim 39 wherein the DNA duplex
binding agent is mitoxantrone.

42. A method for detecting the presence of a target nucleic
acid sequence in a sample, said method comprising:

- 15 (a) adding to a sample suspected of containing said target
nucleic acid sequence, a fluorescently labelled probe specific
for said target sequence, and daunomycin (8S,-cis)-8-acetyl-10-
[3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-
tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacendione),
20 (b) subjecting the thus formed mixture to an amplification
reaction in which target nucleic acid is amplified,
(c) subjecting said sample to conditions under which the said
probe hybridises to the target sequence, and
(d) monitoring fluorescence from said sample.

25